<Division of Biochemistry> Chemical Biology

Division of Biochemistry
- Molecular Clinical Chemistry -

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Scope of Research

This laboratory was founded in 1994 with the aim of linking (bio)chemical research and clinical medicine. Thus, the scope of our research encompasses the structure, function and pathophysiological significance of various biomolecules and bioreactions in relation to human diseases, and the application of molecular techniques to clinical diagnosis and therapy. Our current interest is focused on the role of poly(ADP-ribosyl)ation in protection of genome from apoptosis-inducing stresses, the physiological and pathological functions of brain-specific septin, and the molecular etiology of neurodegenerative disorders including Alzheimer's disease and Parkinson's disease.

Research Activities (Year 2004)

Presentations

Attenuation of Mitochondrial Impairment by Poly(ADP-ribose) Polymerase-1 siRNA in Experimental Models of Cerebral Ischemia and Reperfusion, Tanaka S, Takehashi M, and Ueda K, The 53rd Fujihara International Seminar, Tomakomai, 26 - 29 July.

Suppression of Oxidative Cell Death by Poly(ADP-ribose) Synthetase Inhibitors, Ueda K, Tanaka S, Takehashi M, Banasik M, and Stedeford T, The 53rd Fujihara International Seminar, Tomakomai, 26 - 29 July.

A Pathological Role of CYP2D6 Gene as a Risk Factor for Parkinson's Disease, Tanaka S, Takehashi M, Matoh, N, and Ueda K, 11th Annual Meeting of the Japanese Society for Gene Diagnosis and Therapy, Tokyo, 17 - 18 September.

Attenuation of Mitochondrial Injury by Poly(ADP-ribose) Synthetase siRNA in Experimental Model of Cerebral Ischemia, Tanaka S, Takehashi M, and Ueda K, Joint Meeting of the 27th Annual Meeting of the Japan Neuroscience Society and the 47th Annual Meeting of the Japanese Society for Neurochemistry, Osaka, 21 - 23 September.

Expression, Distribution, and Complex-Formation of Septin 3 Isoforms in Human Brain, Takehashi M, Tanaka S, Tsukagoshi-Nagai H, Kinoshita N, Kawamata T, Ueda K, 77th Annual Meeting of Japanese Biochemical Society, Yokohama, 13 - 16 October.

Poly(ADP-ribose) Polymerase-1 Activation and Mitochondrial Injury Determine the Pattern of Cell Death, Apoptosis or Necrosis, Tanaka S, Takehashi M, and Ueda K, 77th Annual Meeting of Japanese Biochemical Society, Yokohama, 13 - 16 October.

The former professor Kunihiro Ueda retired on 31 March 2004.

A Role of Poly(ADP-ribose) Polymerase-1 in Ischemic Neuronal Cell Death

Poly(ADP-ribose) polymerase-1 (PARP-1), a nuclear enzyme also known as poly(ADP-ribose) synthetase, is activated by DNA strand breaks and forms $(ADP\text{-}ribose)_n$ chains from NAD^+. Ischemic brain injury activates PARP-1 and results in neuronal cell death. A 2-h oxygen-glucose deprivation (OGD) followed by reoxygenation induced apoptosis of rat cortical neurons in culture. Overexpression of a mitochondrial antiapoptotic protein, Bcl-2, efficiently protected the cells from OGD-Reox-induced apoptosis, implying mitochondrial impairment in this process. To support this, the OGD was found to bring about mitochondrial permeability transition (MPT), or membrane depolarization, and a release of proapoptotic proteins from mitochondria. Of the proteins released, cytochrome c was distributed in the cytoplasm and activated a caspase cascade, leading to PARP-1 cleavage in the nucleus. In contrast, apoptosis-inducing factor (AIF) and endonuclease G translocated themselves into the nucleus. Both the MPT and protein translocation were efficiently attenuated by PARP-1-specific inhibitors, 1,5-dihydroxyisoquinoline and benzamide. Knocking down the PARP-1 gene expression with small interfering RNA also protected the cells from apoptotic changes in mitochondria as well as the nucleus. These results indicated a mechanism of ischemia and reperfusion injury in which PARP-1 plays a principal role in inducing mitochondrial impairment, which ultimately leads to apoptosis of neurons.

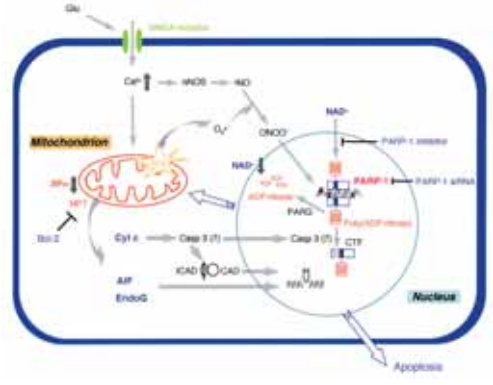

Figure 1. Molecular mechanism of neuronal apoptosis after cerebral ischemia.

Suppression of Chemical-Induced Cell Death by Poly(ADP-ribose) Polymerase-1 Inhibitors

The purpose of the present study was to determine whether 6(5*H*)-phenanthridinone, a potent inhibitor of PARP-1, could attenuate the hepatotoxicity of carbon tetrachloride (CCl_4). Male ICR mice treated via the intraperitoneal route with CCl_4 exhibited severe necrotic centrilobular lesions and significantly elevated serum transaminases. In contrast, the histopathology and serum biochemistry of animals treated concomitantly with CCl_4 and 6(5*H*)-phenanthridinone were not significantly different versus controls. In conclusion, the results of this study demonstrate that the hepatotoxicity of CCl_4 can be blocked independently of its metabolism and suggest the predominant role of PARP-1 overactivation in chemical-induced toxicity.

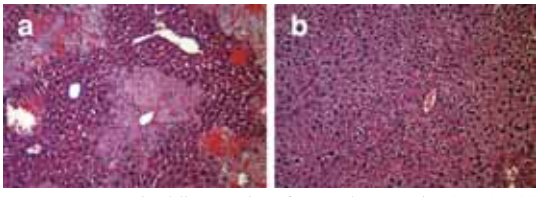

Figure 2. H-E stained liver sections from male ICR mice (200x). (a) CCl_4: severe necrosis in centrilobular regions with loss of hepatocyte morphology and prominent red cell extravasations into the necrotic areas. (b) CCl_4 + 6(5*H*)-phenanthridinone: normal hepatocyte morphology and liver architecture.

Expression, Distribution, and Complex-Formation of Septin 3 Isoforms in Human Brain

Septin 3 is a member of a family of highly conserved 40-60 kDa GTPase-domain proteins called septins. Human *septin 3* was originally cloned as a gene up-regulated upon neuronal differentiation of a human teratocarcinoma cell line NT2. More recently, we found a genetic association of *septin 3* polymorphisms with Alzheimer's disease. Alternative splicing of the *septin 3* gene transcript produces two isoforms, A and B, in the human brain, though their regional expression and physiological function remain to be determined. In the present study, we analyzed the expression patterns of human septin 3 isoforms in normal human brain and a human neuroblastoma cell line, SH-SY5Y, after retinoic acid-induced differentiation. The expression and distribution of septin 3 isoforms A and B were similar and resembled those of another septin, CDCrel-1. Septin 3A and 3B were expressed in normal human brain in a region-specific manner with the highest level in the temporal cortex and hippocampus and the lowest level in the brainstem regions. Prominent immunoreactivity was observed diffusely in the neocortices in association with neuropils and punctate structures suggestive of synaptic junctions. Immunoprecipitation studies revealed that septin 3A, 3B, and CDCrel-1 form a complex in the frontal cortex of human brain. These findings suggest that septin 3A and 3B, along with CDCrel-1, play some important role(s) in synaptogenesis and neuronal development.